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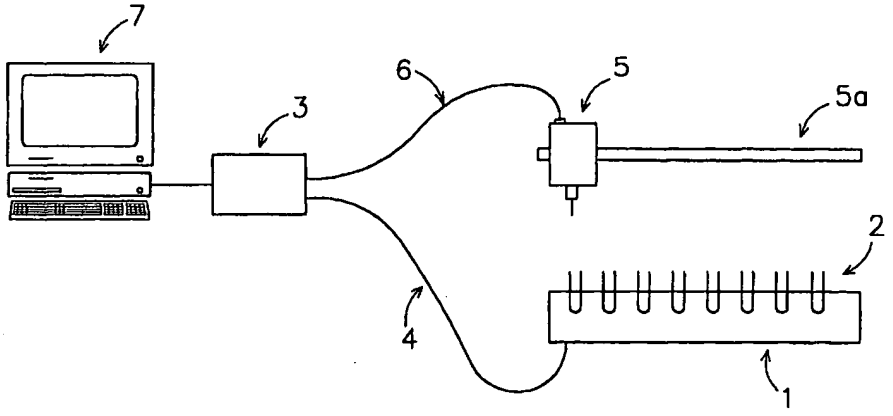
(54) Title: METHOD AND SYSTEM FOR CREATING A LIBRARY OF CROSSLINKED ENZYME AGGREGATES




(57) Abstract: A method for creating a library of crosslinked enzyme aggregates (CLEAs) wherein each CLEA is prepared according to a reaction process comprising an aggregation step of aggregating enzymes and a crosslinking step for crosslinking the enzymes, comprises the steps of creating a basic recipe for a reaction process for preparation of a CLEA, selecting at least one parameter in the basic recipe, which parameter is to be varied, generating a plurality of parameter values, creating a set of recipes comprising a first recipe and at least one second recipe by combing the basic recipe with the plurality of parameter values, the said first and second recipe differing from one another by at least one parameter value, aggregating according to each recipe of the set of recipes, and crosslinking according to each recipe of the set of recipes. Further, the invention comprises a system for executing the method.



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Title: Method and system for creating a library of crosslinked enzyme aggregates.

The invention relates to a method for creating a library of Crosslinked Enzyme Aggregates (CLEAs), also called Crosslinked Enzyme Precipitates, wherein each CLEA is prepared according to a reaction process comprising an aggregation step for aggregating enzymes and a crosslinking step for crosslinking the enzymes. Further, the invention relates to a system for creating a library of Crosslinked Enzyme Aggregates (CLEAs), wherein each CLEA is prepared according to a reaction process comprising an aggregation step for aggregating enzymes and a crosslinking step for crosslinking the enzymes.

From EP 1 088 887 A1, herein incorporated by reference, a process is known for preparing a CLEA, the process comprises two reactions that can be performed in one reaction vessel. First, a solution comprising an enzyme is precipitated using a precipitating agent to form insoluble enzyme aggregates. Second, the insoluble enzyme aggregates are submitted to crosslinking by a crosslinking agent for fixing the structure of the insoluble enzyme aggregates. Precipitation is preferably performed at a temperature between -5° and 100°, more preferably between 0° and 4°C. Further, precipitation is performed in a suitable buffer with a suitable pH, which will usually be between 4-11, the precipitating agent is usually stepwise added over a longer period of time with stirring and pH control when required. The mixture is subsequently allowed to stand for a time, e.g. between 15 min. and 1 hour, then the crosslinking agent is added to the mixture and again left for some time (e.g. between 30 min. and 12 hours). Suitable precipitating agents are in principle all water-soluble precipitating agents that are used in the art of precipitation of bio molecules, suitable crosslinking agents are in principle all agents that can be used in the crosslinking of enzymes.

Despite the many advantages of crosslinked enzyme aggregates, such as that it can be a very simply removed from a mixture and reused without any special separation techniques, CLEAs are still very sporadically used in chemical industrial processes. One of the problems with preparation of an optimal CLEA is that the preparation

of an optimal CLEA is very laborious and time consuming, as chemical properties of the CLEA depend on a large amount of variables, causing the finding of a suitable preparation process for preparing an optimal CLEA for a specific enzyme or for a specific application to be extremely time consuming and to involve a substantial amount of trial and error.

The invention intends to simplify the optimisation process for finding an optimal CLEA for a specific application or for a specific enzyme.

To achieve this goal, the method according to the invention comprises the steps of creating a basic recipe for a reaction process for preparation of a CLEA, the basic recipe at least describing a reactant, the aggregation step and the crosslinking step, selecting at least one parameter in the basic recipe, which parameter is to be varied, the at least one parameter being chosen from a group of parameters relating to the reactant, the aggregation step and the crosslinking step or a combination of two or more thereof, generating a plurality of parameter values for the at least one to be varied parameter, creating a set of recipes comprising a first recipe and at least one second recipe, by combining the basic recipe with the plurality of parameter values, the said first and second recipe differing from one another by at least one parameter value, aggregating according to each recipe of the set of recipes, and crosslinking according to each recipe of the set of recipes. By generating a set of recipes wherein each recipe of the set of recipes differs from another recipe of the set of recipes by at least one parameter value, and preparing a respective CLEA according to each recipe of the set of recipes, a library of CLEAs can be created, from which a suitable CLEA can be easily selected. Further, as the recipes differ from one another by at least one parameter value, an influence of different values of the selected parameter(s) on the CLEAs created can be identified. Of course it will be clear that not only the first recipe and at least one second recipe differ from one another by at least one parameter value, but that the first recipe preferably differs from a plurality of, and most preferably all, second recipes by at least one different parameter value. Thus, each recipe of the set of recipes comprises a different combination of parameter values,

such that each recipe differs from each other recipe of the set of recipes by at least one different parameter value.

The enzyme aggregate can comprise any enzyme (or protein) molecule or particle obtained by any physical technique. The basic recipe comprises a recipe for creating a CLEA, it is possible that the basic recipe comprises parameter values, however it is also possible that in the basic recipe one or more parameter values are left open, and are to be filled in after generating a plurality of parameter values for such parameter. Also it is possible that all parameter values in the basic recipe are left open, and that after selecting the at least one parameter in the basic recipe which is to be varied, all other parameters are filled in with a single value, and that the at least one to be a varied parameter is filled in, for each recipe of the set of recipes, after generating the plurality of parameter values. The library of CLEAs comprises a plurality of CLEAs each CLEA being created according to a recipe of the set of recipes.

Advantageously, the step of generating a plurality of parameter values for the at least one to be varied parameter comprises the steps of defining a range or an increment for varying the at least one parameter value, and generating a plurality of parameter values for the at least one to be varied reaction condition based on the range or increment. The plurality of parameter values can thus be generated easily by, for each parameter, defining a range into which the parameter value is to be varied and/or defining an increment for stepwise increasing or decreasing the parameter value. Thus, a plurality of parameter values is generated by determining parameter values within a range, such as a e.g. equidistant parameter values and/or by determining parameter values making use of an increment such as a step value, for stepwise increasing a parameter value, in combination with e.g. a lowest value, highest value or intermediate value.

Advantageously, at least two parameters are varied, and the step of generating a plurality of parameter values for the at least one to be varies parameter comprises the step of generating a plurality of parameter values for the at least two parameters making use of a statistical analysis. With a statistical analysis, such as a statistical computer program running on a data processing system, such as a computer, a plurality of parameter values for two or more

parameters can be generated in an easy and convenient way. For this purpose, a standard Design Of Experiment (DOE) software can be used. With a suitable statistical analysis a plurality of parameter values for the at least two parameters can be created such that a representative set of recipes each comprising a different combination of parameter values for the at least two parameters, is created.

Advantageously, at least one of the parameters is chosen from a group of parameters influencing an initial condition, the group comprising an enzyme concentration or enzyme dosage, an enzyme surface property, an enzyme purity, an enzyme form and a pH. Thus, parameters which influence an initial condition prior to the reaction can be varied, and thus an effect of a variation in such initial conditions on the CLEA generated by the recipe can be found ~~out~~. Also, a plurality of CLEAs can be created differing from each other by different values of at least one parameter influencing an initial condition, and from the plurality of CLEAs an optimal CLEA can be easily selected.

Likewise, at least one of the parameters is advantageously chosen from a group of parameters influencing the aggregation step, the group comprising a precipitant concentration, a precipitant type, a precipitation time, a precipitant addition speed, an ionic strength, a pH, a temperature, an intensity of stirring, and a stirring time. Thus, an influence of a change of a value of a parameter in the aggregation step on the CLEA created can be retrieved. Also, a plurality of CLEAs can be created differing from each other by different values of at least one parameter influencing the aggregation step, and from the plurality of CLEAs a most suitable CLEA can be easily selected.

Similarly, at least one of the parameters is advantageously chosen from a group of parameters influencing the crosslinking step, the group comprising a crosslinker concentration, a crosslinker adding speed, a crosslinker type, a crosslinking time, a type of quenching agent, an addition speed of the quenching agent, a dosage of quenching agent, a molar ratio of crosslinker to enzyme, a pH, an ionic strength, a temperature, an intensity of stirring, and a stirring time, for monitoring an influence of a change in value of a parameter influencing the crosslinking reaction on the CLEA, and for creating a plurality of CLEAs differing from each other by different

values of at least one parameter influencing the crosslinking step, and selecting a suitable CLEA from the plurality of CLEAs. By adding the quenching agent (such as an amine, preferably an amino acid, such as glycine), the crosslinking time can be accurately set as the addition of the quenching agent will quench the crosslinking process.

Advantageously, the reaction process comprises an addition step for adding an additive, the basic recipe describing the addition step. The additive preferably is a crown ether, a surfactant, a cofactor, or an analogue thereof. The addition step can be performed before, during or after the aggregation step or the crosslinking step, or can be comprised in any of these steps. The addition step is carried out according to each recipe of the set of recipes.

Advantageously, step b) comprises the step of b1) selecting at least one parameter relating to the addition step. Thus, a plurality of CLEAs can be created differing from each other by different values of at least one parameter influencing the addition step, and from the plurality of CLEAs a most optical CLEA can be easily selected. Thus, the parameter selected in step b) can be chosen from a group of parameters relating to the reactant, the aggregation step, the crosslinking step, the addition step, or a combination of two or more thereof.

Advantageously, at least one of the parameters is chosen from a group of parameters influencing an additive condition, the group comprising a type of additive, an additive concentration, an additive dosage, an additive form and a pH.

It is also possible that the at least one parameter is chosen from a group of parameters influencing two or more of a group comprising the initial condition, the aggregation step, ~~and~~ the crosslinking step and the addition step, such as an overall temperature. Further it is possible that two or more parameters being varied, of which one parameter influences one of the initial condition, the aggregation step and the crosslinking step, and of which another parameter influences another one of the initial condition, the aggregation step and the crosslinking step. Further, of course other parameters can also be varied. Varying the parameters nor only provides insight in a relation between a variation of the parameter and the CLEA, but also enables to quickly, advantageously

in parallel, create a large amount of CLEAs of which a most suitable CLEA can be selected.

Advantageously, the step of aggregating according to each recipe of the set of recipes and the step of crosslinking according to each recipe of the set of recipes are performed in parallel for all recipes of the set of recipes. This reduces the time to create the CLEAs. Also, the addition step can be performed in parallel for all recipes of the set of recipes. Further, usage of an automated laboratory system can be made, wherein the basic recipe, the selection of at least one parameter to be varied, the generation of a plurality of parameter values and the creation of the set of recipes can be performed making use of suitable software. After creation of the recipes, suitable instructions can be sent to equipment comprised in the automated laboratory system for executing each recipe.

Advantageously, the method comprises the further step of isolating each CLEA from a respective reactor. The isolation of the CLEA can e.g. be performed by centrifugation or any other suitable separation technique, such as drying or filtration. It is for example possible that the reactor in which the aggregation step and the crosslinking step have been performed is transferred to a centrifuge for separating the CLEAs.

The system according to the invention comprises any suitable means for executing the method according to the invention.

Further features and advantages of the invention will become clear from the appended drawing in which a non-limiting embodiment of the invention have been described, in which:

Fig. 1 shows a system for creating a library of CLEAs according to the invention; and

Fig. 2 shows a method for creating a library of CLEAs according to the invention.

The system shown in Fig. 1 comprises a holder 1 for holding a plurality of reaction vessels 2. In each reaction vessel 2 a CLEA is created by means of a reaction process comprising an aggregation step for aggregating enzymes and a crosslinking step for crosslinking the enzymes. Therefore, the holder 1 comprises a stirring means (not shown) for stirring a substance in each reaction vessel 2 and temperature conditioning means (not shown) for conditioning a temperature of a substance in each reaction vessel 2. The stirring

and temperature conditioning can be controlled individually for each reaction vessel 2, and therefore the holder 1 comprises separate temperature conditioning means and separate stirring means for each reaction vessel 2. The holder 1 is connected to a data processing system 3 via a data link 4. The system further comprises a dosing robot 5 for providing substances to the reaction vessels 2. Therefore, the dosing robot 5 is provided with a transportation means 5a for displacement of the dosing robot 5 or part thereof, from one reaction vessel to another one of the reaction vessels 1. The dosing robot 5 is controlled by the data processing system 3 via a data link 6 between the data processing system 3 and the dosing robot 5. It will be clear that the dosing robot 5, which might be known in the art per se, has been depicted highly schematically and can have any suitable shape, dimensions and construction. The data processing system 3 is connected to a personal computer 7 or any suitable terminal for inputting and/or outputting data. By means of the personal computer 7, a basic recipe can be created, parameters selected and parameter values entered, as will be explained below with reference to Fig. 2. The reaction process can further comprise an addition step for adding an additive before, during or after any of the aggregation step and the crosslinking step. The addition can be performed by e.g. the dosing robot 5.

In Fig. 2, in step 10 a basic recipe is created. The basic recipe can be created by a user making use of personal computer 7. The basic recipe can be entered making use of a suitable program, wherein e.g. elementary recipe operations from a library are combined into a basic recipe. Also it is possible that the basic recipe is created with the aid of a computer in many other ways, e.g. making use of pictograms, selection menu's, or any other data entry method known in the art. The basic recipe describes at least a reactant, the aggregation step and the crosslinking step. Further, the basic recipe can describe an addition step for adding an additive. Then, in step 11 at least one parameter in the basic recipe is selected. The parameter, which is to differ between at least two of the reactions taking place in the plurality of reaction vessels 2, is chosen from a group of parameters comprising the reactant, the aggregation step and the crosslinking step or a combination of two or more thereof. Also, the parameter can be chosen from the addition step. Selecting the

parameter can be performed manually, i.e. by a person selecting or entering the parameters in any suitable way in the personal computer 7, or can be performed automatically by the personal computer 7 or by the data processing system 3. Next, in step 12 a plurality of parameter values for the at least one to be varied parameter is generated. This can be performed by defining a range or an increment for varying the at least one parameter followed by a generation of the plurality of parameter values based on the range or increment. Then, in step 13, a set of recipes is created by combining the basic recipe with the plurality of parameter values. Step 12 and 13 can be performed by the personal computer 7, or by the data processing system 3 or by a combination of both. Once the set of recipes is created, a recipe is available for creating a CLEA in each reaction vessel 2 of the plurality of reaction vessels 2. Each recipe of the set of recipes differs from each other recipe of the set of recipes by at least one different value of a parameter. Thus, a recipe can differ from an other recipe by a different value of a first parameter, while the recipe can differ from still an other recipe by a different value of a second parameter. Also it is possible that recipes differ from each other by different values of two or more parameters. Now that the set of recipes has been created, a set of instructions for executing each recipe is created in the data processing system 3. Then, an aggregation step 14 is performed in each reaction vessel 2 of the plurality of reaction vessels 2. Next, a crosslinking step 15 according to each recipe of the set of recipes is performed in each of the reaction vessels 2. Further, an addition step (not shown) can be performed according to each recipe of the set of recipes. The addition step can be performed before, during or after any of the aggregation step and the crosslinking step, or can be comprised in any of these steps.

Thus, in each reaction vessel 2 of the plurality of reaction vessels 2, a CLEA is created according to a recipe of the set of recipes. As each recipe of the set of recipes differs from each other recipe of the set of recipes by at least one parameter value, a library of CLEAs is created, each CLEA of the library of CLEAs being created according to a different recipe of the set of recipes. Now that a library of CLEAs is available, an optimum CLEA for a specific application can be easily selected from the library of CLEAs making

use of any suitable selection technique. The library of CLEAs can be created by a plurality of reaction processes performed simultaneously or consecutively in the plurality of reaction vessels, however it is also possible that several "batches" of reactions are performed after each other, for creating a library of CLEAs comprising a number of CLEAs higher than the number of parallel reaction vessels 2 comprised in the plurality of reaction vessels 2. Thus, when the plurality of reaction vessels comprised in the holder 1 comprises 50 reaction vessels, and the library of CLEAs is to comprise 500 CLEAs each created according to a different recipe, 10 batches of reaction processes are to be performed. By selecting all parameters which might have any influence on the properties of the CLEA, and varying values for these parameters (i.e. applying different values of these parameters for different recipes), a plurality of CLEAs can be created which differ from each other by different values of the parameters for the recipes according to which the respective CLEAs are created. The parameters can influence an initial condition, i.e. a condition before start of the aggregation step, such as an enzyme concentration, also, the different values of the parameters can result in different reaction conditions in the aggregation step, in the crosslinking step and/or the addition step. Of course it is also possible that one or more parameters influence two or more of the group comprising the initial condition, the aggregation step, the crosslinking step, and preferably the addition step.

After creation of the CLEAs in the plurality of reaction vessels 2, the CLEAs can be separated from the mixture by means of any suitable separation technique, such as by centrifugation. Therefore, the reaction vessels 2 are removed from the holder 1 and placed into a suitable separation means, such as a centrifuge.

The reactions according to the different recipes taking place in the plurality of reaction vessels 2 can take place in parallel, i.e. simultaneously, however it is also possible that the reaction processes in the reaction vessels 2 are performed partly parallel, partly sequential, or in any other time relation to each other.

The personal computer 7 and the data processing 3 can both be placed on the same location, however it is also possible that the personal computer 7, or any other terminal, is positioned on a remote location and connected to the data processing system 3 via a network,

such as an intranet or an internet. Alternatively it is possible that the personal computer 7 and the data processing 3 are integrated into a single unit, i.e. in that the functions of the data processing system 3 are performed by the personal computer 7 or any suitable hardware means, such as an extension card inserted into the personal computer. The datalink 4 and the datalink 6 can comprise any suitable data connection comprising any suitable protocol, point to point connection and/or network. The dosing robot 5 can be of any suitable type for providing the appropriate reactant(s), precipitant(s), crosslinker(s), quenching agent(s), and preferably additive(s) etc. to each reaction vessel 2 of the plurality of reaction vessels, according to the respective recipes.

# CLAIMS

1. A method for creating a library of Crosslinked Enzyme Aggregates (CLEAs), wherein each CLEA is prepared according to a reaction process comprising an aggregation step for aggregating enzymes and a crosslinking step for crosslinking the enzymes, the method comprising the steps of:

a. creating a basic recipe for a reaction process for preparation of a CLEA, the basic recipe at least describing a reactant, the aggregation step and the crosslinking step;
b. selecting at least one parameter in the basic recipe, which parameter is to be varied, the at least one parameter being chosen from a group of parameters relating to the reactant, the aggregation step and the crosslinking step or a combination of two or more thereof;
c. generating a plurality of parameter values for the at least one to be varied parameter;
d. creating a set of recipes comprising a first recipe and at least one second recipe, by combining the basic recipe with the plurality of parameter values, the said first and second recipe differing from one another by at least one parameter value;
e. aggregating according to each recipe of the set of recipes; and
f. crosslinking according to each recipe of the set of recipes.

2. The method according to claim 1, characterised in that step c. comprises the steps of:
c1. defining a range or an increment for varying the at least one parameter value; and
c2. generating a plurality of parameter values for the at least one to be varied reaction condition based on the range or increment.

3. The method according to claim 1 or 2, characterised in that at least two parameters are varied, and in that step c comprises the step of generating a plurality of parameter values for the at least two parameters making use of a statistical analysis.

4. The method according to any of the preceding claims, characterised in that at least one of the parameters is chosen from a group of parameters influencing an initial condition, the group comprising an enzyme concentration or enzyme dosage, an enzyme surface property, an enzyme purity, an enzyme form and a pH.

5. The method according to any of the preceding claims, characterised in that at least one of the parameters is chosen from a group of parameters influencing the aggregation step, the group comprising a precipitant concentration, a precipitant type, a precipitation time, a precipitant addition speed, a pH, an ionic strength, a temperature, an intensity of stirring, and a stirring time.

6. The method according to any of the preceding claims, characterised in that at least one of the parameters is chosen from a group of parameters influencing the crosslinking step, the group comprising a crosslinker concentration, a crosslinker addition speed, a crosslinker type, a crosslinking time, a type of quenching agent, an addition speed of the quenching agent, a dosage of quenching agent, a molar ratio of crosslinker to enzyme, a pH, an ionic strength, a temperature, an intensity of stirring, and a stirring time.

7. The method according to any of the preceding claims, characterised in that the reaction process comprises an addition step for adding an additive, the basic recipe describing the addition step.

8. The method according to claim 7, characterised in that step b) comprises the step of b1) selecting at least one parameter relating to the addition step.

9. The method according to claim 8, characterized in that at least one of the parameters is chosen from a group of parameters influencing an additive condition, the group comprising a type of additive, an additive concentration, an additive dosage, an additive form and a pH.

10. The method according to any of the preceding claims, characterised in that step e) and f) are performed in parallel for all recipes of the set of recipes.

11. The method according to any of the preceding claims, characterised in that the method comprises the further step of isolating each CLEA from a respective reactor.

12. A system for creating a library of Crosslinked Enzyme Aggregates (CLEAs), wherein each CLEA is prepared according to a reaction process comprising an aggregation step for aggregating enzymes and a crosslinking step for crosslinking the enzymes, the system comprising:

a. means for creating a basic recipe for a reaction process for preparation of a CLEA, the basic recipe at least describing a reactant, the aggregation step and the crosslinking step;
b. means for selecting at least one parameter in the basic recipe, which parameter is to be varied, the at least one parameter being chosen from a group of parameters relating to the reactant, the aggregation step and the crosslinking step or a combination of two or more thereof;
c. means for generating a plurality of parameter values for the at least one to be varied parameter;
d. means for creating a set of recipes comprising a first recipe and at least one second recipe, by combining the basic recipe with the plurality of parameter values, the said first and second recipe differing from one another by at least one parameter value;
e. means for aggregating according to each recipe of the set of recipes; and
f. means for crosslinking according to each recipe of the set of recipes.

13. The system according to claim 9, characterised in that means for generating a plurality of parameter values comprises:
c1. means for defining a range or an increment for varying the at least one parameter value; and

c2. means for generating a plurality of parameter values for the at least one to be varied reaction condition based on the range or increment.

14. The system according to claim 12 or 13, characterised in that at least two parameters are varied, and in that the means for generating a plurality of parameter values comprise: means for generating a plurality of parameter values for the at least two parameters making use of a statistical analysis.

15. The system according to any of claims 12-14, characterised in that the means for aggregating and the means for crosslinking are arranged for aggregating and crosslinking in parallel for all recipes of the set of recipes.

16. The system according to any of claims 12-15, characterised in that the system further comprises:
means for isolating each CLEA a the respective reactor.

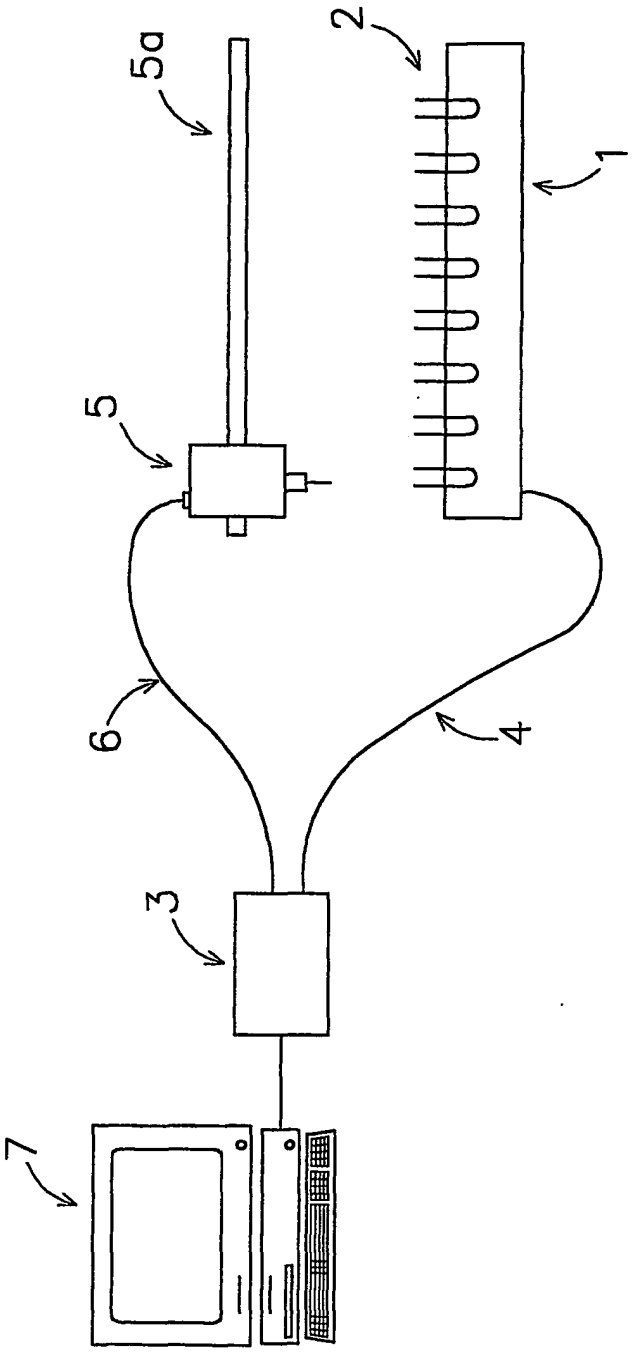


Fig 1

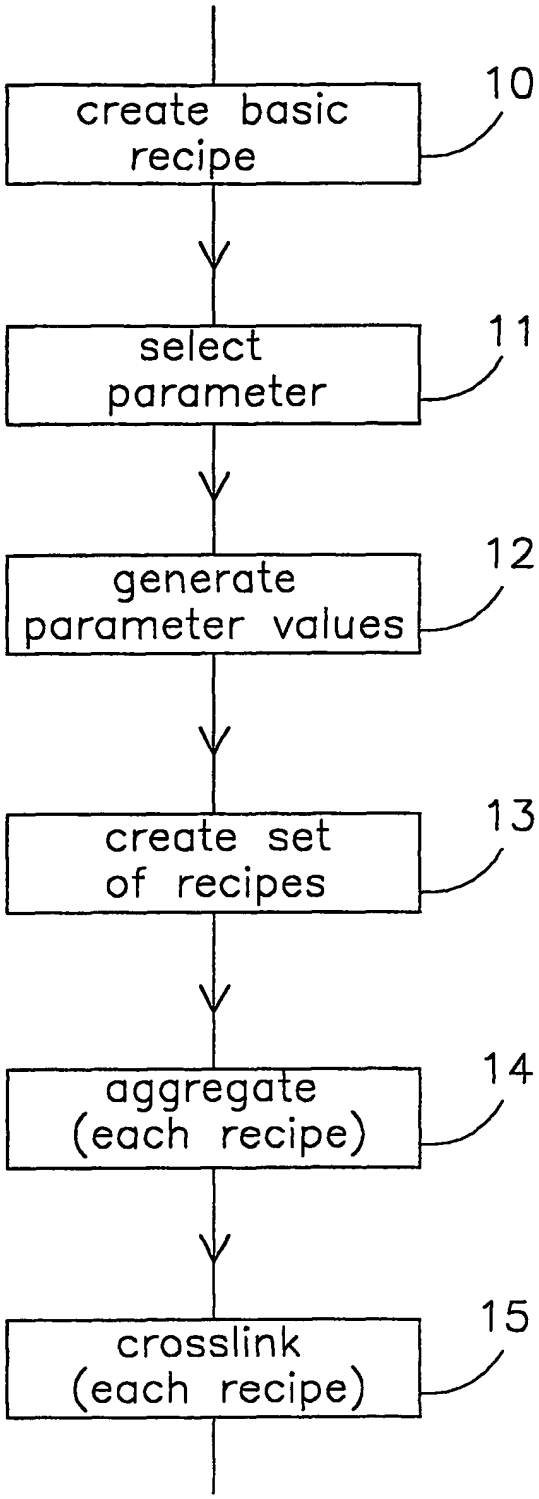
create basic recipe
10
select parameter
11
generate parameter values
12
create set of recipes
13
aggregate (each recipe)
14
crosslink (each recipe)
15

Fig 2

# INTERNATIONAL SEARCH REPORT

PCT 02/00720

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/96

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 1 088 887 A (DSM NV)<br>4 April 2001 (2001-04-04)<br>cited in the application<br>paragraph '0009! - paragraph '0011!;<br>examples<br>--- | 1-16 |
| X | CAO L ET AL: "Cross-linked enzyme aggregates: a simple and effective method for the immobilization of penicillin acylase"<br>ORGANIC LETTERS, ACS, WASHINGTON, DC, US,<br>vol. 2, no. 10, 18 May 2000 (2000-05-18),<br>pages 1361-1364, XP002202388<br>ISSN: 1523-7060<br>page 1362, left-hand column, last line<br>-right-hand column, last line; figure 2<br>---<br>-/-- | 1-16 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
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| 1 July 2003 | 11/07/2003 |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | CAO L ET AL: "CROSS-LINKED AGGREGATES OF PENICILLIN ACYLASE: ROBUST CATALYSTS FOR THE SYNTHESIS OF BETA-LACTAM ANTIBIOTICS" JOURNAL OF MOLECULAR CATALYSIS. B, ENZYMATIC, ELSEVIER, AMSTERDAM,, NL, vol. 4-6, no. 11, 22 January 2001 (2001-01-22), pages 665-670, XP001079507 ISSN: 1381-1177 page 666, right-hand column, paragraph 3; tables 1,2,4 --- | 1-16 |
| X | LOPEZ-SERRANO P ET AL: "CROSS-LINKED ENZYME AGGREGATES WITH ENHANCED ACTIVITY: APPLICATION TO LIPASES" BIOTECHNOLOGY LETTERS, KEW, SURREY, GB, vol. 24, no. 16, August 2002 (2002-08), pages 1379-1383, XP009003775 ISSN: 0141-5492 page 1379 -page 1380; tables 1,2 --- | 1-16 |
| A | WO 00 40331 A (VANERDEN LYNN ;DALES CAMERON G (US); NIELSEN RALPH B (US); SAFIR A) 13 July 2000 (2000-07-13) abstract; figures 4,7 page 13, line 5 -page 14, line 28 ----- | 12-16 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/ 02/00720

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP 1088887 A | 04-04-2001 | EP 1088887 A1 | 04-04-2001 |
| WO 0040331 A | 13-07-2000 | AU 2722700 A | 24-07-2000 |
| | | AU 3742599 A | 25-10-1999 |
| | | EP 1062033 A1 | 27-12-2000 |
| | | EP 1310791 A2 | 14-05-2003 |
| | | EP 1308722 A2 | 07-05-2003 |
| | | EP 1310789 A2 | 14-05-2003 |
| | | EP 1308723 A2 | 07-05-2003 |
| | | EP 1018002 A2 | 12-07-2000 |
| | | JP 2002501622 T | 15-01-2002 |
| | | WO 0040331 A1 | 13-07-2000 |
| | | WO 9951980 A2 | 14-10-1999 |
| | | US 2003086822 A1 | 08-05-2003 |
| | | US 6536944 B1 | 25-03-2003 |
| | | US 6508984 B1 | 21-01-2003 |

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